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DETERMINATION OF ANTI-DIABETIC ACTIVITY OF GYNURA PROCUMBENS USING BIOASSAY –GUIDED FRACTIONATION

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By

SITI PAULIENA BINTI MOHD BOHARI

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirement for the Degree of Master of Science

July 2006

DEDICATION

My beloved parents (Mak and Abah)

My sisters and brothers

My Grandfather

Thanks for your prayers, support, encouragement, motivation and sacrifice…….

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of requirement for the degree of Master of Science

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Faculty : Faculty of Biotechnology and Biomolecular Sciences

This study aims to investigate the antidiabetic and hypoglycaemic properties of Malaysian herb, *Gynura procumbens* (Sambung nyawa). Bioassay guided fractionation has been carried out to identify the bioactive crude fraction responsible for antidiabetic activity of *Gynura procumbens*. Both *in vitro* and *in vivo* model study were used to evaluate the antidiabetic properties of this plant.

In vitro insulin secretion study, glucose uptake study and cytotoxicity were used as primary assay on crude methanolic extract, hexane, ethyl acetate and butanol fractions. Cytotoxicity studies demonstrated that crude methanolic extract have the lowest cytotoxicity when compared with crude fractions *Gynura procumbens* in BRIN BD11 cell lines. Cytotoxicity study with adipocytes and muscle cell lines showed that the crude methanolic extract of *Gynura procumbens* have the lowest toxicity when

compared with the crude fractions of the plant. Determination of insulin secretion response was done by using BRIN BD11 cell lines and from the result, it showed that crude hexane and ethyl acetate crude fractions have good potential in stimulation of insulin release. Glucose uptake study with adipocytes cell lines (3T3 mouse adipocytes cell lines) indicated that this plant has the dose dependent manner and *Gynura procumbens* crude hexane fraction indicated the highest activity on stimulating glucose uptake. Effect of crude methanolic extract and crude fractions in the presence of insulin showed moderate glucose uptake activity when compared with *Gynura procumbens* crude extract/ fractions alone. Glucose uptake study with a mouse L6 muscle cell lines indicated that *Gynura procumbens* crude methanolic extract has highest reading from all of the crude extracts. When comparing the crude extract and fractions with insulin, all of the results showed moderate glucose uptake activity and thus expressed that this plant has dose dependent manner.

Further study was done with Type 1 and Type 2 model diabetic rats. It shows that crude methanolic extract of *Gynura procumbens* have hypoglyceamic activity on both models. This result was further investigated with gut perfusion study using crude methanolic extract and ethyl acetate fraction of *Gynura procumbens* and showed positive result by delaying glucose absorption in the rat intestine.

Further investigation was done with ethyl acetate fraction that showed potential activity from *In vitro* and *In vivo* study. Identification of compounds by using reverse phase HPLC showed some promising peaks of interesting compounds. Isolation and

purification was carried on by using various chromatography techniques such as normal chromatography and gel filtration Sephadex LH-20. Two compounds from isolated from ethyl acetate fraction were mix of β-sitosterol and stigmasterol, and kaemferol-3-O- glucoside.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

PENGENALPASTIAN AKTIVITI ANTI-DIABETES *GYNURA PROCUMBENS* MENGGUNAKAN BIOASAI BERDASARKAN FRAKSINASI

Oleh

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Kajian ini bertujuan untuk mengkaji ciri-ciri antidiabetes dan hipoglisemik daripada pokok herba Malaysia iaitu *Gynura procumbens* (Sambung nyawa). Teknik pengasingan biocerakinan berpanduan telah digunakan untuk mendapatkan sebatian daripada tumbuhan ini. Untuk menilai ciri-ciri antidiabetes daripada tumbuhan ini, model pegkajian secara *in vivo* dan *in vitro* telah digunakan.

Penyelidikan secara *in vitro* menggunakan asai perembesan insulin, asai pengambilan glukosa dan asai sitotoksik sebagai penaksiran awal bagi ekstrak kasar metanol, fraksi heksana, fraksi etil asetat dan fraksi butanol. Penyelidikan sitotoksik menunjukkan bahawa ekstrak kasar metanol menunjukkan ketoksikan terendah apabila dibandingkan dengan fraksi-fraksi yang lain dalam jujukan sel BRIN BD11. Penyelidikan ketoksikan dengan menggunakan jujukan sel adipos and sel otot menunjukkan bahawa ekstrak metanol *Gynura procumbens* menunjukkan toksik terendah. Penentuan respon perembesan insulin telah dijalankan dengan menggunakan jujukan sel BRIN BD11 dan

keputusan menunjukkan bahawa fraksi heksana dan etil asetat menunjukkan potensi yang bagus dalam mengaruh rembesan insulin apabila dibandingkan dengan kawalan. Penyelidikan pengambilan glukosa dengan jujukan sel adipos (sel 3T3 adipos tikus) menandakan pokok ini memiliki ciri kebergantungan kepada dos ekstrak atau fraksi itu sahaja. Fraksi heksana daripada *Gynura procumbens* memberikan aktiviti tertinggi dalam pengaruhan pengambilan glukosa apabila dibandingkan dengan kawalan (tanpa rawatan). Kesan pada ekstrak metanol dan semua fraksi dengan kehadiran insulin hanya menunjukkan aktiviti yang sederhana jika hendak dibandingkan dengan ekstrak dan fraksi-fraksi *Gynura procumbens* sahaja. Penyelidikan pengambilan glukosa dengan sel L6 otot tikus menunjukkan ekstrak metanol *Gynura procumbens* mengaruh pengambilan glukosa dan menunjukkan bacaan tertinggi apabila dibandingkan dengan ekstrak metanol dan semua fraksi tumbuhan ini. Apabila dibandingkan ekstrak bersama insulin, hanya menunjukkan aktiviti yang sederhana dan menunjukan bahawa pokok ini memiliki ciri kebergantungan kepada dos dari tumbuhan itu sahaja.

Kajian diteruskan dengan model tikus diabetes. Kajian menunjukkan bahawa ekstrak metanol *Gynura procumbens* mempunyai aktiviti hipoglisemik terhadap kedua-dua jenis model (Diabetes kategori 1 dan Diabetes kategori 2). Penyelidikan ini diikuti dengan 'Gut perfusion' eksperimen yang menggunakan fraksi etil asetat sahaja. Daripada keputusan yang diperolehi menunjukkan keputusan yang memberangsangkan dengan melambatkan penyerapan glukosa ke dalam usus kecil tikus.

Penyelidikan diteruskan lagi dengan fraksi etil asetat yang telah menunjukkan aktiviti yang memberangsangkan selepas diuji dengan '*In vitro study*' dan '*In vivo study*'.

Pengenalpastian sebatian dengan menggunakan HPLC 'reverse phase' telah menunjukkan beberapa kemuncak sebatian yang menarik. Pengasingan dan penulenan dilakukan dengan menggunakan pelbagai jenis teknik kromatografi seperti kromatografi normal dan filtrasi gel sephadex LH-20. Dua sebatian telah berjaya diasingkan iaitu campuran β-sitosterol dan stigmasterol dan kaempferol-3-O- glucoside .

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I certifiy that an Examination Committee has met on 17 July 2006 to conduct the final examination of Siti Pauliena Binti Mohd Bohari on her Master of Science thesis entitled "Determination of Anti-Diabetic Activity of *Gynura procumbens* using Bioassay-Guided Frcationation" accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follow:

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DECLARATION

I hereby declare that the thesis is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that is has not been previously or concurrently submitted for any other degree at UPM or other institutions.

_____

SITI PAULIENA BINTI MOHD BOHARI

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LIST OF ABBREVIATIONS

β	Beta
BIRDEM	Bangladesh Institute of Research & Rehabilitation in Diabetes, Endocrine and Metabolic Disorder
BSA	Bovine serum albumin
b.w	Body weight
^{13}C	Carbon 13
CO_2	Carbon dioxide
cc	Column chromatography
δ	Chemical shift in ppm
$CHCl_3$	Chloroform
cm^2	Centimeter square
˚ C	Degree Celsius
DMSO	Dimethyl sulfoxide
d	Doublet
dd	Doublet of doublet
DEPT	Distortionless Enhancement by polarization Transter
ELISA	Enzyme-Linked Immunosorbent Assay
EtoAC	Ethyl acetate
FBS	Feotal bovine serum
g	Gram
GDM	Gestational diabetes mellitus

GLUT4	Glucose transporter 4
HMBC	Heteronuclear Multiple Bond Correlation
Hz	Hertz
HPLC	High Performance Layer Chromatography
hr	Hour
IC_{50}	Concentration of 50% inhibition
IDDM	Insulin dependent diabetes mellitus
Kg	Kilogram
L	Litre
M	Molar
MeOH	Methanol
µl	Microlitre
mg	Miligram
NIDDM	Non insulin diabetes mellitus

CHAPTER 1

INTRODUCTION

Diabetes mellitus is a metabolic disorder in the endocrine system. The disease is one of the major public health concerns and is rapidly increasing in most parts of the world. People suffering from diabetes are not able to produce or properly use insulin in the body, so they have a high content of blood glucose.

As a very common chronic disease, diabetes is becoming the third 'killer' along with cancer, cardiovascular and cerebrovascular diseases because of its high prevalence, morbidity and mortality (Li *et al*., 2004). The cause of diabetes is a mystery, although both genetic and environmental factors such as obesity and lack of exercise appear to play a role. Ethnic and racial differences have been found in heterogenous populations within the same area. Most researchers believe that in the presence of a genetic predisposition, something in the environment triggers the development of the diabetes. With a long cause and serious complications often resulting in high death-rate, the treatment of diabetes spent vast amounts of resources including medicines, diets, and physical training in all countries. Up to now, many kinds of antidiabetic medicines have been developed for patients, but almost all are chemical or biochemical agents. The fact is that there has been no report of someone recovering totally from diabetes (Li *et al*., 2004).